AMENDED VERSION

IN THE SPECIFICATION:

Beginning on page 6, line 26:

Cytotrophoblasts, retinal pigment epithelial cells, and Sertoli cells all express indoleamine 2,3-dioxygenase (IDO), a tryptophan catabolizing enzyme, that appears to be critical in maintenance of maternal tolerance (Munn et al., 1998). In pregnant mice treated with 1-methyl-tryptophan, an inhibitor of IDO, rapid T-cell mediated rejection of all allogeneic pregnancies occurred. Syngeneic pregnancies of mice treated with the same inhibitor were not affected (Munn et al., 1998). The expression of IDO is regulated in human cells by interferons (Malina and Martin, 1996); the most efficient of these is interferon-~~?~~γ IFN-~~?~~γ. Interferons have anti-cancer activity and can inhibit tumor cell growth in culture (Taylor and Feng, 1991). It has been shown *in vitro* that a primary mechanism of the cytotoxicity of IFN-~~?~~γ is the induction of IDO. IDO uses two superoxide radicals to cleave the pyrrole ring of tryptophan, an essential amino acid, in the first, and rate limiting step of tryptophan catabolism, and is an antioxidant enzyme (Malina and Martin, 1996). It is now well established that tryptophan starvation resulting from IFN-~~?~~γ treatment is the mechanism of the antiproliferative activity of IFN-~~?~~γ on many cell lines and intracellular parasites (Taylor and Feng, 1991). Tryptophan starvation can lead to apoptosis of cell. Within 48 h of treatment with IFN-~~?~~γ ME180 human epidermoid carcinoma cells underwent apoptosis that could be prevented by adding tryptophan and induced by removing it in the absence of IFN-~~?~~γ. Replication of the parasite *Toxoplasma gondii* was inhibited by treatment of infected RPE cells in culture with IFN-~~?~~γ. The inhibition could be reversed by addition of tryptophan (Nagineni et al., 1996).

Beginning on page 60, line 19:

Measurement of immune response to grafted cells. This section describes the measurement of host response to grafted cells, as in Example 5. Grafts that are rejected show dense mononuclear cell infiltration, pronounced expression of CD25 and an upregulation of several cytokines including IL-2, IL-4, IFN-~~2~~γ (Lehman et al., 1997). Therefore, measurement of the presence of cells producing these cytokines is a method of determining the immune response to the implanted cells. Immune events are followed in the graft by using quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistology. Cytokine gene expression is performed as described (Siegling et al., 1994). Total RNA is prepared from biopsies of each graft and reverse transcribed into cDNA. The cytokine gene expression is quantified using a control fragment which contains primer sequences of rat cytokines and β-actin and HPRT (Lehman et al., 1997). A constant amount of sample cDNA is mixed with varying known amounts of competitor fragment to compete for amplification with specific primers. Proportions of PCR fragments amplified from control fragment and target cDNA are estimated after separation on a 1.5% agarose gel by measuring the intensity of ethidium bromide luminescence with a CCD image sensor. Data is analyzed using the EASY program (Herolab, Weisloch, Germany). cDNA samples are adjusted according to the β-actin and HPRT housekeeping gene signals and the gene expression of T cell markers (CD 3 and CD 25) and cytokines are quantified using the competitive RT-PCR amplification of the target cDNA. Values are expressed in arbitrary units (AU). An AU is the lowest concentration of control fragment that yields a detectable product with one specific primer pair (Lehman et al., 1997).

Beginning on page 70, line 20:

Isolation of Sertoli Cells. Sertoli cells are isolated from the testes of 15-21 day-old male rat pups (either Sprague Dawley or Lewis strains) following

the protocol described by Korbutt *et al.*, (Korbutt et al., 1997). Briefly, wet the rat body with ethanol and open the abdominal cavity. Remove the tunica from each testes and place in a tube containing 1X HBSS. Remove excess solution and the outer connective tissue. Weigh the tissue and chop into finer pieces. Transfer to 50-ml tube containing 45 ml of 1X Hank's Balanced Salt Solution (HBSS), 5 ml of trypsin solution (1.25 mg/ml) and 100 ?µl of DNase (6.64 mg/ml). Incubate at 37°C for 25 min with occasional but gentle swirling. Allow the tissue to settle and aspirate the supernatant. Add 5 ml of trypsin inhibitor and allow settling for 1-2 min. Wash with Hanks (3X). After the final wash add 5 ml of collagenase (0.7 mg/ml), 5 ml of HBSS and 50 ?µl of DNase (6.64 mg/ml). Than add 10 ml of HBSS and incubate at 37°C for 15 min with occasional swirling. Spin at 2,000 RPM for 2 min. Wash in the same way twice again. Resuspend pellet in F-12 Ham's medium (without serum) containing 100U/ml penicillin, 100 ?g/ml streptomycin and plate on tissue culture plates. Incubate at 37°C in a 5% CO_2 incubator. Fetal bovine serum (FBS) is added to the medium at 10% concentration and cells are propagated in that medium. We have successfully isolated, propagated, and grown the cells from frozen stocks.

Staining Sertoli cells with antibody to follicle-stimulating hormone receptor (FSHr). Lewis Sertoli cells (1-2 x 10^4) are plated into each well of an 8-well Nalge Nunc Lab-Tek Chamber Slide, and then covered and grown in a CO_2 incubator at 37°C for 2 days. The cells are fixed with 50:50 acetone:ethanol solution at 4°C for 10 min and the solution aspirated and the cells allowed to air day. The sample is stored in a 50:50 mix of PBS:glycerol at 4°C. The PBS:glycerol solution is removed and the samples washed twice with PBS at RT for 5 min just prior to staining. To block nonspecific binding of secondary antibodies the slide is incubated in PBS with 10% normal rabbit serum, 0.2% Triton X-100, and 0.1% bovine serum albumin (BSA) at RT for 3 h. This solution is removed and the slide blocked by PBS with 2% normal rabbit serum, 0.2% Triton X-100, and 0.1% BSA (2% rabbit-TX-BSA) at RT for 10 min. The

primary antibody (Ab; sheep Ab to FSHr; Biogenesis, Brentwood, NH) (Korbutt et al., 2000) is diluted with 2% rabbit-TX-BSA from stock (7.2 mg/ml) to give a final concentration of 2 ?µg/ml, 4 ?µg/ml or 8 ?µg/ml in each well. After adding the diluted Ab the plate is incubated at 4°C overnight. The primary Ab is removed, and the plate is washed three times with PBS at RT for 5 min. The secondary Ab (biotinylated rabbit anti-goat IgG) is diluted 1:150 in PBS and incubated with the slide at RT for 60 min. The slide is washed three times with PBS at RT for 5 min. Then the slide is incubated with avidin conjugated Alexa Fluor 350 (1:1000 dilution in PBS; Molecular Probes, Oregon) at RT for 1 h. This is followed by three PBS washes, and then the slide is allowed to air day and after adding mounting medium (Vectashield, Vector Labs, Burlingame, CA) a coverslip is placed on it. Fluorescence is evaluated at 200 X magnification, using a Nikon Optiphot microscope equipped with an epi-fluorescence attachment (Figure 11) in conjunction with SPOT 1 digital camera (Diagnostic Instruments, Sterling Heights, MI) and Photoshop 6.0 (Adobe, San Jose, CA).

Injections of cells into spinal cord. Before implantation, the cells are rinsed twice with PBS, trypsinized and resuspended in serum free medium at a concentration of 1 x 10^5 cells/?µl. The cells are implanted close to the site of injury with a 32-guage beveled needle with a 45-degree angle 2 inches in length (Hamilton # 0160832). The needle is attached to a 10-?µl Hamilton syringe and a Harvard apparatus. Before implanting, an incision are made with 30-guage needle and the new needle placed into the incision position. Cells are implanted at a rate of 0.2 ?µl/min. A total of 2 ?µl are implanted and needle left in for an additional 5 min. Animals are sutured and kept in the recovery chamber till they gain consciousness.

Beginning on page 73, line 10:

Tissue processing for analysis. Animals are re-anesthetized and euthanized at 42 days after injury. Animals are perfused with 4% paraformaldehyde in 0.1 M PBS. Spinal cord of about 2 cm is isolated and is

divided into a minimum of three segments, corresponding to the site of contusion and 1 cm proximal and distal to the contusion. The tissue is post-fixed for 4 h, cryoprotected in 20% sucrose for 3 to 4 days and stored at -70°C in OCT medium. 14-?µm thick transverse sections are made on a cryostat and collected onto Fisher SuperFrost Plus slides. Sections are stored at -70°C and are analyzed as described below. Every 5th section is used for each of the analysis and the rest of the sections are stored as a library for any further staining needed.

Beginning on page 75, line 5:

Viral infection. To enable long term NT-3 expression by the cells, they are infected with a replication-deficient adenovirus that expresses eGFP that is obtained from the Gene Transfer Vector Core facility, University of Iowa, Iowa City, IA. The protocol is as follows: 20,000 cells (Sertoli) are plated per well of the 8 well chamber slide a day before the infection. The stock viral concentration is 1 x 10^{12} particles/ml or 1 x 10^{9} particles/?µl. Perform serial dilutions to give 1 x10^{8} particles/?µl, 1 x 10^{7} particles/?µl and 1 x 10^{6} particles/?µl. Cells are infected at 100 particles/cell, 1,000 particles/cell, 10,000 particles/cell and 100,000 particles/cell. Control cells are not infected with any virus. Before infection the cells are washed three times with serum free medium (SFM) and incubated with the respective viral concentration at 37°C for 4 hours. At the end of the infection period add serum-containing medium and continue incubation at 37°C. Cells are analyzed 3 and 6 days post infection by fluorescence microscopy. The number of cells infected increased with the number of virus particles used per cell. At 10,000 particles per cell most of the cells are infected, but at the higher concentration there is cell death. Hence, following this experiment the cells are infected at 10^{4} viral particles/cell.

Beginning on page 76, line 19:

Implantation and survival of syngeneic cells into the rat spinal cord. Laminectomy is performed on male Sprague Dawley rats to expose the T8 disc of the spinal cord. Sertoli cells (isolated from Sprague Dawley pups) are

infected with the virus Ad5GFP, 24 hours before implantation. Cells are implanted with a 32-gauge needle hooked to a Hamilton syringe and a Harvard apparatus. Before implanting, an incision is made with 30-gauge needle and the new needle is placed into the incision position. Cells that are harvested are resuspended at a concentration of 1 x 10^5 cells/?µl and are implanted at a rate of 0.2 ?µl/min. A total of 2 ?µl is implanted and needle is left in for an additional 5 min.

Beginning on page 78, line 5:

a: Embryonic neuronal cultures. Embryonic cortical neurons are isolated from mouse fetuses on embryonic day 16.5. These cells are cultured *in vitro* and the bioactivity of NT-3 secreted in the supernatant of the Sertoli cells infected with adenovirus is tested. Purified human NT-3 (BioVision, Mountain View, CA) and dehydroepiandrosterone (DHEA) are used as positive controls for the assay. Bioactivity is tested by measurements of the length of the neurites. The assay is performed as described by (Compagnone and Mellon, 1998). Cortical hemispheres are separated from the midbrain and hindbrain, and the basal ganglia are removed. After the removal of the hippocampus and the meninges, the cortical tissue is cut into small pieces and placed in PBS containing 0.03% collagenase and 1 ?µg/ml DNase I for 30 min at 37°C. After the incubation, a single cell suspension is made by mechanical trituration with a spinal needle and cells are filtered through a 40-?µm nylon mesh. Cells are plated (50,000 cells per cm^2) on glass coverslips coated with poly-D lysine (5 ?µg/cm^2, Roche) and 10% dextran and charcoal treated fetal bovine serum (Hyclone; Logan, UT). The culture media is a modification of N2 serum-free medium used for culturing neuroblastoma cell lines (Bottenstein and Sato, 1979). The medium is DMEM-Ham F12 1:1 (2.24 g/liter bicarbonate, no phenol red) without serum, containing glucose (3.15 g/l), L-glutamine (2 mM), insulin (5 ?µg/ml, Roche), transferrin (5 ?g/ml, Roche), selenium (3 X 10^{-8} M; Roche), putrescine (10^{-4} M; Sigma Chemical Co, St. Louis, MO), and lipids (0.5 ?µl/ml, GIBCO/BRL; Rockville, MD). Cells are allowed to settle and attach to the coverslip for 2 h before the coverslip is inverted, as described (Lucius and Mentlein, 1995). Sandwiched cells are cultured for 3 days in 5% CO_2 at 37°C and then treated according to the conditions

described above. All the treatments are done in triplicates and the cells are treated for 16-20 h.

Beginning on page 85, line 18:

The isolation protocol is as follows. The derivation of TS cell lines from 3.5*dpc* mouse blastocysts is similar to the derivation of embryonic stem (ES) cell lines (Kuehn et al., 1987; Labosky et al., 1994; Tanaka et al., 1998). Briefly, matings are set up between mice of interest. Prepare 4-well plates of mitomycin-treated primary embryonic fibroblasts (EMFIs) in medium (RPMI 1640 that contains 20% fetal bovine serum, penicillin/streptomycin (5 ?µg/ml, each), sodium pyruvate (1mM), beta-mercaptoethanol (100 ?µM) and L-glutamine (2 mM) the day before flushing. Replace TS medium with TS+F4H (FGF4, Sigma; and heparin) medium in the morning of the flushing day. Flush and collect 3.5dpc blastocytes. In sterile conditions place one blastocyst per well in the 4-well plates containing TS+F4H medium and culture at 37°C/5%CO_2. The blastocytes should hatch and attach to the wells in 24-36 hrs. Feed culture with TS + F4H medium. Disaggregate the outgrowth on day 4 or 5 of culture as follows. Remove the medium and wash with PBS. Aspirate and add 0.1% trypsin/EDTA and incubate at 37°C/5%CO_2 for 5 min. Disaggregate the clump by pipetting up and down gently. Immediately stop the trypsinization by adding 70% conditioned medium (TS medium harvested from mitomycin C treated EMFIs) + 30% TS medium + 1.5x F4H. Change the medium 8 hr after disaggregation. Feed cells regularly and passage half-confluent well of TS cells to a regular 6-well plate or 35mm dish or into plates that contain mitomycin C treated MEFs. Most of the cells are frozen after the first passage, the remaining are used for the studies. As described below, cell morphology is the guideline for the number of the times the cells are passaged in culture. In culture normally three cell types are seen. Stem cells are the least differentiated and form the major population of the culture. Intermediate and giant cells are the differentiated forms of the stem cells and form a minor population of the total culture. Cells are cultured *in vitro* only till most of them are undifferentiated.

Beginning on page 87, lines 9:

Treatment of effectors with mitomycin. To prevent proliferation the effector cells are treated with a solution of mitomycin C (10 ?µg/ml in culture media) at 37°C for 2 h. Then the cells are washed 3 times with PBS to remove the mitomycin C. The trophoblast cell cultures are trypsinized, and washed. The effector cells are counted, and resuspended in RPMI 1640 media as described above (6,000 cells in 100 ?µl of RPMI 1640 media, 6 x 10^4 cells/ml). Then 100 ?µl is added per well of a 96-well flat-bottomed microtiter plate, and the plate incubated in a CO_2 incubator at 37°C for 4 hours.

Beginning on page 88, line 1:

The CD1 responder spleen cells are labeled with BrdU according to the instructions of the vendor. Briefly, the cells are adjusted to 2 x 10^5 cells/ml of culture medium. Next, the BrdU labeling solution from the kit is added to the cells to a final concentration of 10 ?µm, and the cells incubated for 15 hours at 37°C. The cells are centrifuged at 250 x *g* for 10 min. The supernatant is carefully removed and the cells are suspended in BrdU-free RPMI culture media. Then 10,000 spleen responder cells labeled with BrdU are added in 100 ?µl in RPMI media (1 x 10^5 cells/ml) per well of a 96-well microtiter plate containing 6,000 mitomycin C treated effector cells per well and the plate is incubated for 6 hours in incubator at 37°C. To 4 wells the responder cells are added as described to wells containing media without effector cells and as a positive control an aliquot of dexamethasone is added to achieve a final concentration of 25 nm. Dexamethasone induces apoptosis in T lymphocytes with an ED_{50} of 10 nM (Perandones et al., 1993). Responder cells are also added to 8 wells containing only supernatant from the progenitor trophoblast or Sertoli cell cultures. The microtiter plate is centrifuged at 300 x *g* for 10 min and 100 ?µl of supernatant is carefully removed from each well and analyzed for DNA labeled with BrdU by ELISA. The detection of BrdU labeled DNA in the supernatant is indicative of the release of DNA fragments from damaged target cells and is reflective of cell-mediated cytotoxicity.

Beginning on page 88, line 26:

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Beginning on page 90, line 12:

Preparation of rat spleen cells. Rats are anesthetized with a mixture of ketamine/xylazine (66mg/kg, 6.6mg/kg, respectively) given intramuscularly. After the anesthetic takes effect a midline incision is made extending to the posterior to expose the abdominal cavity. After locating the spleen, the surrounding tissue is dissected with forceps, and the spleen gently removed and rinsed with 70% ethanol. The animal is sacrificed by cervical dislocation. The spleen is placed in a cell culture dish on ice and accessory

tissue removed. Cold PBS is added to the dish and the spleen is cut into small pieces. The spleen cells are isolated by repeated aspiration in cold PBS using a syringe with an 18-gauge needle. The resulting cell suspension are filtered through several layers of sterile surgical gauze to remove large clumps, and then through a 70-micron sterile cell nylon. The cell culture dish is rinsed with cold PBS and the rinse liquid filtered also. The suspension is centrifuged for 7 - 10 min at 300 x *g* in a 15-ml centrifuge tube. Then it is diluted to 10 to 15 ml and aspirated with pipette to distribute the cells evenly. Clumps that will not disperse are discarded. Centrifugation and wash step are repeated as needed up to 3 times. The cells are cultured in RPMI 1640 with 5% rat or fetal bovine serum and penicillin/streptomycin antibiotics at 37°C in 5% CO_2. To count viable cells 10 ~~?~~µl of cell suspension at 10^7 cells/ml are mixed with 10 ~~?~~µl of Trypan blue solution (0.2% of Trypan blue in PBS wth 3 mM NaN_3) in a small tube and resuspended with a pipette tip. The cells are examined in a hemacytometer chamber scoring more than 100 cells as to their state of viability within 5 min. Blue cells are dead and unstained are live. The red blood cells are lysed in isotonic ammonium chloride solution as described in example 11 above.~~—~~